WARMTH
WORLD ASSOCIATION OF RADIOPHARMACEUTICAL AND MOLECULAR THERAPY
FOUNDED 2009

International Symposium on Radiopharmaceutical Therapy
Helsinki, 18-20 November 2018

DAY 1, SUNDAY, NOVEMBER 18, 2018

13.00 – 18.30	REGISTRATION for ISRT 2018
14.00 – 16.00	**SCIENTIFIC SESSION 1:** NUCLEAR CARDIOLOGY Moderators: Juhani Knuuti, Turku, Finland and Pietro Muto, Naples, Italy
14.00 – 14.30 O1	**Revolution in cardiac imaging: Quantitative perfusion, inflammation and hybrid imaging** Juhani Knuuti, Turku, Finland
14.30 – 15.00 O4	**Novel therapies of cardiac regeneration. How imaging can help in diagnosis, targeting and monitoring?** Seppo Ylä-Herttuala, Kuopio, Finland
15.00 – 15.30 O2	**Imaging innervation. Finally clinical applications?** Albert Flotats, Barcelona, Spain
15.30 – 16.00 O3	**Gauging cardiac repair, regeneration and inflammation with new molecular probes** James Thackeray, Hannover, Germany
16.00 – 16.30	**COFFEE & TEA BREAK**
16.30 – 18.45	**SCIENTIFIC SESSION 2:** NEW TRENDS IN ONCOLOGY AND PRECISION MEDICINE. Moderators**: Vivek Subbiah**, MDACC, Houston, TX, USA and **Homer Macapinlac**, MDACC, Houston, TX, USA
16.30 – 16.55 O5	**Personalized medicine and precision oncology,** Vivek Subbiah, Investigational Cancer Therapeutics, UT MD Anderson Cancer Center
16.55 – 17.20 O6	**Precision oncology in rare tumors: adopting the orphans,** Roman Groisberg, Division of Medical Oncology, Rutgers Cancer Institute of New Jersey, New Brunswick, NJ, USA
17.20 – 17.45 O7	**Optimizing patient selection for phase 1 clinical trials: lessons from targeted therapy and immunotherapy drug development,** Shiraj Sen, Drug Development, Sarah Cannon Research Institute at HealthOne; Denver, CO, USA

17.45 – 18.20 O8	**Target discovery for precision oncology using large public databases,** Jason Roszik, Department of Genomic Medicine, UT MD Anderson Cancer Center
18.20 – 18.45 O12	**Re-188 Lipiodol Liver Cancer Project – an update on current status,** Ajit Shinto, Coimbatore, India

WARMTH CONGRESS DINNER 1

19.30 – 22.30 Restaurant Meripaviljonki

DAY 2, MONDAY, NOVEMBER 19, 2018

08.00 – 18.00	REGISTRATION for ISRT 2018
09.00 – 11.00	**OPENING CEREMONY**
Welcome Address	Partha Choudhury, President-Elect, WARMTH
Opening Remarks	Kalevi Kairemo, Congress President, ISRT-2018, WARMTH
Welcome Address	Pirkko Mattila, Minister of Health, Finland
WARMTH Candle Lighting Ceremony	WARMTH Board Members
AJIT PADHY ORATION	Steven M. Larson, MSKCC, NY, USA
O10	"**New Insights in Theragnostics**"
presenter	Andrew M. Scott, Melbourne, Australia
11.00 – 11.30	**COFFEE & TEA BREAK**

11.30 – 13.00	**SCIENTIFIC SESSION 3:** RADIOEMBOLISATION AND LIVER THERAPIES
	Moderators: Patrick Flamen, Brussels, Belgium and Aviral Singh, Bad Berka, Germany

11.30 – 11.55 O11 **Personalized SIRT based on predictive dosimetry, regional functional reserve measurement and molecular imaging**, Patrick Flamen, Jules Bordet Institute, Brussels, Belgium

11.55 – 12.20 O9 **Oncolytic viruses,** Akseli Hemminki, University of Helsinki

12.20 – 12.40 O13 **Biologic dosimetry in SIRT**, Katherine Vallis, University of Oxford, UK

12.40 – 13.00 O14 **Intra-arterial PRRT of SSTR-expressing liver tumors**, Aviral Singh, Bad Berka, Germany

Posters: P1 ***The quantitative analysis of post-selective internal radiation therapy (SIRT) ^{90}Y microspheres PET/CT in hepatocellular carcinoma in comparison with ^{99m}Tc-labelled macroaggregated albumin (MAA) planar and SPECT/CT,*** Ngoc Ha Le, Tran Hung Dao Hospital, Ho Chi Minh City, Vietnam

13.00 – 14.00 **LUNCH AT THE CONGRESS CENTER PAASITORNI**

14.00 – 16.00 **SCIENTIFIC SESSION 4:** NEW RADIONUCLIDE THERAPIES / RADIATION HAZARDS/ SAFETY ISSUES
Moderators: Seigo Kinuya, Kanazawa, Japan and Kazuko Ohno, Fukushima, Japan

14.00 – 14.30 O15 **Boron neutron capture therapy and world's first accelerator-based BNCT facility at Southern Tohoku General Hospital,** Yoshihiro Takai, Southern Tohoku BNCT Research Center, Japan

14.30 – 15.00 O16 **What did we learn from the Fukushima accident?** Ohtsura Niwa, Radiation Effects Research Foundation, Hiroshima/ Nagasaki, Japan

15.00 – 15.30 O17 **Regulatory nuclear safety aspects, Linear No-Threshold Hypothesis of risk from low-level radiation exposure,** Bennett S Greenspan, Society of Nuclear Medicine and Molecular Imaging, USA

PANEL-discussion: Regulatory/ nuclear safety aspects

Moderators: Seigo Kinuya, Kazuko Ohno and Bennet S Greenspan

Posters: P2 ***Establishment of incident reporting programme and lesson learnt in unsealed radionuclide therapy,*** Noreen Marwat, Nuclear Medicine Oncology and Radiotherapy Institute, Pakistan

P3 ***Practices across Pakistan how medical emergency is handled in case of patients who has undergone unsealed radionuclide therapy,*** Noreen Marwat, Nuclear Medicine Oncology and Radiotherapy Institute, Pakistan

16.00 – 16.30 **COFFEE & TEA BREAK**

16.30 – 18.15 **SCIENTIFIC SESSION 5:** *PROSTATE CANCER: PSMA RADIOLIGAND THERAPY (PRLT) – WHAT DO WE KNOW AND WHAT IS NEW?*
Moderator: Richard P. Baum, Bad Berka, Germany

16.30 – 16.45 O18 **Introduction to PRLT,** Richard P. Baum

16.45 – 17.00 O19 **Dosimetry following Lu-177 PSMA radioligand therapy and an insight into novel nadionuclides for theranostics of prostate cancer,** Aviral Singh, Bad Berka, Germany

17.00 – 17.15 O20 **Dosimetry in PSMA radioligand therapy of metastasized prostate cancer using Lu-177 PSMA I&T and Lu-177 PSMA-617** Christiane Schuchardt, Bad Berka, Germany

17.15 – 17.45 O21 **PSMA targeting and therapy trials in Australia,** Andrew M. Scott, Melbourne, Australia

17.45 – 18.00 O22 **Ac-225- and Bi-213- PSMA-617 radioligand therapy in patients with castration resistant prostate cancer**, Mike Sathekge, Pretoria, South Africa

18.00 – 18.15 O23 **[^{18}F]AIF-PSMA-HBED-CC and ^{177}Lu-PSMA-617 as a potential theragnostic tandem and comparison with 68Ga-PSMA-HBED-CC in high-risk prostate cancer patients at initial staging**, Omar Alonso, CUDIM, Montevideo, Uruguay

Posters: P4 ***^{177}Lutetium-prostate-specific membrane antigen radionuclide treatment of lymph node metastatic prostate cancer with PSA recurrence: A cohort study***, Finn Edler von Eyben, Odense, Denmark

P5 ***Dosimetry in Molecular Radiotherapy - Bad Berka Experience***, Christiane Schuchardt, Bad Berka, Germany

P6 ***Web-Monitoring Tool for ^{177}Lutetium-PSMA Treatments in Prostate Cancer Patients***, Kalevi Kairemo, Helsinki, Finland

18.25 *Tram transportation to the City Hall in front of the Scandic Paasi hotel (Hakaniemi Square stop 0252)*

WARMTH CONGRESS DINNER 2

19.00 – 20.30 **CITY HALL of Helsinki, Mayor Reception**

20.45 -21.45 *Tram transportation from the City Hall (Market Square stop) to the Scandic Paasi hotel (Hakaniemi Square) including sightseeing in Helsinki*

DAY 3, TUESDAY, NOVEMBER 20, 2018

08.00 – 08.45 REGISTRATION for ISRT 2018

8.45 – 11.00 **SCIENTIFIC SESSION 6:** THYROID CANCER – QUO VADIS? Moderators: Mark Tulchinsky, PA; USA & Raihan Hussain, Dhaka, Bangladesh

Debating Controversies in Radioiodine Imaging and Therapy of Thyroid Cancer

8.45 – 9.00 O24 **Introduction-thyroid cancer management,** Mark Tulchinsky, Hershey, PA, USA

9.00 – 9.15 O25 **Total vs. subtotal thyroidectomy. East, West , what´s the best?** Ilya V. Sleptsov, St. Petersburg, Russia

9.15 – 9.30 O26 **Dosimetric approach of thyroid cancer**, Henry Bom, Chonnam, South Korea

9.30 – 9.45 O27 **Management of I-131 refractory thyroid cancer: a multimodality approach**, Partha Choudhury, New Delhi, India

9.45 – 9.55 O28 **Prevalence of genetic duet and its influence on the prognosis of differentiated papillary thyroid carcinoma patients**, Sanjana Ballal, New Delhi, India

9.55 – 10.00 O29 **Comments -thyroid cancer management**, Raihan Hussain, Dhaka, Bangladesh

Panel discussion: Moderator Mark Tulchinsky

Panelists: Raihan Hussain, Henry Bom, Partha Choudhury and Ilya Sleptsov, St. Petersburg, Russia

10.05 – 10.30		**"Radioiodine Imaging Before, After, Both or Neither … and How?"**
10.30 – 10.55		**"Controversies in Radioiodine Side-Effects: Confusion About Salivary Damage and Secondary Malignancy"**
Posters:	P7	***Antithyroglobulin antibody as a marker of successful ablation therapy in differentiated thyroid cancer,*** Ayu Rosemeilia Dewi, Universitas Padjadjaran, Indonesia
	P8	***Importance of isolated raised thyroglobulin antibody in follow up and management of differentiated thyroid cancer***, Ray Soumendranath, Tata Medical Center, Kolkata, India
	P9	***Contribution of manual fusion in thyroid cancer whole body study with I-131. Case Report***, Mariela Agolti, Parana, Argentina
	P10	***Contribution of manual fusion in thyroid cancer whole body study with I-131,*** Mariela Agolti, Parana, Argentina
11.00 – 11.10		Lifetime Achievement Awards & Group Photo Irene Virgolini, President, WARMTH & Kalevi Kairemo
11.10 – 11.30		COFFEE & TEA BREAK
11.30 – 13.00		**SCIENTIFIC SESSION 7:** PEPTIDE RECEPTOR/ NEW THERAPIES Moderator: **Irene Virgolini, Innsbruck, Austria**
11.30 – 11.45 O30		**Value of FDG in NET: importance of dual tracer imaging,** Margarida Rodrigues Radischat, Innsbruck, Austria
11.45– 12.00 O31		**Value of Re-PRRT in NET,** Anna Yordanova, Bonn, Germany
12.00– 12.15 O32		**PRRT in G3-NEN,** Aviral Singh, Bad Berka, Germany
12.15– 12.35 O33		**New peptides for PRRT in Non-NETs,** Irene Virgolini, Innsbruck, Austria
12.35– 12.50 O34		**Long-term side effects and quality of life, patients view** Josh Mailman, San Francisco, USA

12.50– 12.58 O35 **A correlation between c-Fos expression and radioiodine in breast cancer cell lines**, Aisyah Elliyanti, Andalas University/ Dr.M.Djamil Hospital, Indonesia

Posters: P11 ***First ex-vivo experience with I- radiation and radioguided surgery technique in meningioma and neuroendocrine patients***, Chiara Maria Grana, European Institute of Oncology, Milan, Italy

P12 ***Lutetium-labelled DOTA-TOC and radionuclide therapy (PRRT) in China: First Experience***, Feng Wang, Nanjing, China

P13 ***Developing neuropeptide Y (NPY) nanoconstructs as potential theranostic agents***, Irfan Ullah Khan, Institute of Nuclear Medicine & Oncology, Lahore, Pakistan

13.00 – 14.00 **LUNCH AT THE CONGRESS CENTER PAASITORNI**

14.00 – 16.00 **SCIENTIFIC SESSION 8:** NEW ALPHA- THERAPIES
Moderators: Oyvind Bruland, Oslo, Norway and Roy Larsen, Oslo, Norway

14.00– 14.30 O36 **Predicting the future of Alpha: clinical indications and radioisotopes of choice?** Jean-Francois Chatal, Arronax, Nantes, France

14.30– 14.50 O37 **Radiometabolic therapy with ^{223}Ra-dichloride: the European Institute of Oncology experience**, Chiara Maria Grana, European Institute of Oncology,Milan, Italy

14.50– 15.10 O38 **Ra-223 in osteosarcoma –Ph I-trial**, Vivek Subbiah, MD Anderson Cancer Center, USA

15.10– 15.30 O39 **Ra-224 labelled biodegradable carbonate microparticles (Radspherin^{R}) to combate microscopical residual peritoneal carcinomatosis**, Tina Bønsdorf, Oncoinvent AS, Oslo, Norway

15.30– 15.50 O40 **"Dual-alpha" - an expanding technology for development of targeted alpha therapies**, Asta Juzeniene, Norwegian Radium Hospital, Oslo, Norway

Posters: P14 ***Clinical case of benefit due to deviation in Ra-223 treatment schedule***, Tatiana Kochetova, MRRC, Obninsk, Russia

P15 ***Personalized molecular imaging with bone scintigraphy, NaF-PET, and FDG-PET for evaluation of osteosarcoma response to Radium-223***, Kalevi Kairemo, Houston, TX, USA

P16 ***Treatment response evaluation in soft-tissue osteosarcoma metastases using fluoride-18 (^{18}F)-PET/CT radiomics analysis for ^{223}Ra-therapy***, Kalevi Kairemo, Houston, TX, USA

P17 ***Substantiation of an individual therapeutic dose of ^{153}Sm-oxabiphor for the treatment of bone metastases***, Ganna Grushka, Grigorev Institute for Medical Radiology, Ukraine

P18 ***Radionuclide therapy for bone pain palliation in anemic patients: role of erythropoietin***, Sukanta Barai, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India

P19 ***^{18}F-fluoroethyltyrosine PET/CT: the European Institute of Oncology experience in brain tumors***, Chiara Maria Grana, European Institute of Oncology, Milan, Italy

P20 ***Pharmaceutical development of the therapeutic radiopharmaceutical based on –emitting Sm-153 in heat-sensitive carrier for brachytherapy of tumors of various locations***, NM Tolbit, Karpov Institute, Obninsk, Russia

15.50 – 16.20 COFFEE & TEA BREAK

16.20 – 17.00 **SCIENTIFIC SESSION 9:** FUTURE ASPECTS OF COLLABORATION OF WARMTH, IAEA AND WFNMB – ROUND TABLE DISCUSSION
Moderator: Irene Virgolini, Innsbruck, Austria

WARMTH perspective, Partha Choudhury, President-Elect

IAEA Perspective, NN, Vienna

WFNMB perspective, Andrew M. Scott, Melbourne, Australia

SNMMI perspective, Bennett Greenspan, USA

O41 Presentation of ICRT 2019, Feng Wang, Nanjing, China

O42 Closing remarks, Kalevi Kairemo

A Correlation between c-Fos Expression and Radioiodine in Breast Cancer Cell Lines

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Abstract

Objective: This study investigated c-Fos expression in two breast cancer cell lines (ER+/PR+ and HER2+ subtypes) and analysed toxic effect of radioiodine on those cells based on c-Fos expression for possibility of using radioiodine as breast cancer adjuvant therapy, particularly for patients who are resistance with existing therapy.

Materials and Methods: This study uses breast cancer cell lines (MCF7 and SKBR3), and keratinocyte cell line (HaCaT) as control. To induce c-Fos expression, the cells were treated with epidermal growth factor (EGF) 50ng/ml, Adenosine tri-phosphate (ATP) 100μM and a combination of both for twenty-four hours and this was followed by 7.4 x 10_5 Becquerel/well of radioiodine (NaI-131). A quantitative real-time reverse transcriptase polymerase chain reaction (qRT-PCR) and immunocytofluoresence were used to assess c-Fos expressions.

Results: It was found that c-Fos expression is only found in MCF-7 cells. A combination of ATP and EGF has the potential to induce a 23 ±4 fold ($p<0.05$) increase of c-Fos mRNA and the values for ATP and EGF treatments were 1.17 ± 0.02 and 5 ± 2 respectively. Treatment of EGF or a combination of ATP and EGF reduces the reproductive ability of MCF-7 cell up to 100% after radioiodine exposure ($p<0.05$). It was found that inverse correlation between both c-Fos mRNA and protein expression with radioiodine effect on reproductive ability with r = - 0.90 and r = - 0.97 ($p<0.05$) respectively**.**

Conclusion: There is a strong inverse correlation between the expressions of c-Fos with the effect of radioiodine in breast cancer cell. C-Fos expression is involved in cell death pathways after radioiodine exposure in MCF7 cells, while other pathway may correspond to cell death in SKBR3 cells.

Keywords*:* Cell death, epidermal growth factor, adenosine tri-phosphate, sodium iodide symporter, reproductive ability

I. INTRODUCTION

Breast cancer is the most commonly occurring cancer worldwide, with more than one million cases diagnosed annually and about 600,000 fatalities [1]. Breast cancer is a heterogeneous disease; thereby tumors at a similar stage and with similar histopathology can

have diverse biological behavior and resulting responses to treatment [2,3]. Breast cancers are classified into five main subtypes; luminal A, luminal B, HER2, basal and normal like, according to the expression of hormone receptors [4]. Luminal A has a low recurrence score, and responds well to endocrine therapy. Luminal B has high recurrence score and is less responsive to endocrine therapy. However, the effectiveness of endocrine therapy has limitation, and around 30% of patients with Luminal A [5]. On the other side HER2+/neu subtype responds poorly to current chemotherapies [2]. New treatments, including radioiodine (^{131}I) therapy are being developed to increase prognosis of breast cancer patients.

Radioiodine has been used for many decades in management thyroid cancer as adjuvant therapy after near-total thyroidectomy [6,7]. ^{131}I is a radionuclide which emits ionizing radiation. It emits two types of radiation, gamma (γ) and beta particle (β-) radiation, which are used to diagnose and treat a number of diseases [8]. Radiation of radioiodine has low *linear energy transfer* (LET), 0.25 KeV/μm approximately, and its penetration into soft tissue is about 1mm. A physical phase interaction between a β particle and the cell leads to cell destruction by direct and indirect mechanism. When radiation hits a DNA chain, the molecular structure can be change directly leading to cell death. A radiation can also hits cytoplasm cell which consists of almost 70% water to forms hydroxyl radicals. The free radicals in cytoplasm result the cell damage and caused cell death indirectly [8-12]. Free radicals induce cell cycle arrest by prolonging chromatin binding of phospho- extracellular signal-regulated kinase (ERK) and induce c-Fos transcription and regulates phosphorylated at C-terminal sites in chromatin. As a result, Fra-1 fails to attach to chromatin, and causing the cell to fail to express cyclin D1 and enter the S-phase of cycle cell [13].

Ionizing radiation activates several signal pathways inside the cells and can induce either the death of the cell or cell proliferation [8,14]. In breast cancer cells which have an estrogen receptor α (ERα), the expression of E2F1 dependent on c-Fos plays a role in cell proliferation, differentiation and apoptosis [15]. Luminal A is breast cancer subtypes responses to hormonal therapy. However, Luminal A subtype is reported have a recurrence of the disease after being treated with tamoxifen around 30% of patients [5]. Furthermore, HER2+ subtype is reported has poorly response to current chemotherapies [2]. New therapy modalities are needed to be developed especially for those showed resistance. In this study we assessed c-

Fos expression in two breast cancer cell lines (ER+/PR+ and HER2+ subtypes) and analysed toxic effect of radioiodine on those cells based on c-Fos expression for possibility of using radioiodine as breast cancer adjuvant therapy, particularly for patients who are resistance with existing therapy.

II. MATERIAL AND METHODS

Cell lines and culture condition

This study uses two types of breast cancer cell lines MCF7 (estrogen and progesterone positive receptors/ ER+ and PR+) and SKBR3 (human epidermal growth factor receptor 2/ HER2+) [16,17]. HaCaT cell line (a normal keratinocyte cell) as cell control. SKBR3 was supplied by the American Type Culture Collection (ATCC), MCF7 and HaCaT cell lines were gifted from the Faculty of Medicine, Universitas Padjadjaran, Bandung-Indonesia. MCF7 and HaCaT cells were cultured in a RPMI 1640 medium (Sigma-Aldrich). SKBR3 was cultured in McCoy's 5A medium (Sigma-Aldrich). All mediums were supplemented with 10% fetal bovine serum (Sigma-Aldrich), 1% Penicillin, 1% Streptomycin and 1% Amphotericin B. The cells were incubated at 37_oC and supplied with 5% carbon dioxide (CO_2) until 80% confluence was reached. To induce c-fos expression, the cells were incubated in serum-free medium overnight and then treated with EGF 50ng/ml (Abcam #ab9697), ATP 100 µM (Sigma-Aldrich # 1388), and a combination of EGF and ATP for 30, 45 and 60 minutes. Cells which did not receive any treatment after culturing were used as treatment controls.

Quantitative Real-time reverse transcriptase –PCR (qRT-PCR)

The treated and untreated cells were harvested at an appropriate time by trypsinization. The cells were washed with PBS 2 twice and divided into PCR tube 4.10_5 cells/tube. The cell centrifuged at 1000 rpm for 4 minutes and storage at -80_oC. The total RNA was isolated from the cells using a RNeasy mini kit (Qiagen #74106) following the manufacturer's instructions. Product C-Fos RNA was quantified using Nanodrop 2000. Five 5 ng of RNA

reversely transcribed and analysed by one step real-time quantitative PCR using Rotor Gene Quantitect probe RT-PCR (Qiagen # 204443). The synthesis composition was quantitec probe RT MM 12.5 µL, primer F 1µL, Primer R 1µL, Probe 1µL, quantitec RT mix 0.25 µL, RNAase free water 6.25µL, and RNA 3µL. Annealing temperature 60_oC and amplified for 40 cycles. *C Fos forward: GCG GAC TAC GAG GCG TCA T. The reverse: GGA GGA GAC CAG AGT GGG C. Probe: CTC CCC TGT CAA CAC ACA GGA CTT TTG C. β-actin forward*: ACC GAG CGC GGC TAC AG. *The reverse*: CTT AAT GTC ACG CAC GAT TTC C. *Probe*: TTC ACC ACC ACG GCC GAG C. The c-Fos expression of treated cells to those untreated ones was analyzed using the method ($2^{-\Delta\Delta CT}$) described by Livak et al [18]. qPCR assays were conducted in triplicate.

Immunocytofluoresence

The cells were seeded on coverslips in wells of a 24-well culture plate ($2.5\ 10_5$ cells /well). The treated and untreated cells were then rinsed three times with phosphate buffer saline (PBS) and fixed using 4% paraformaldehyde for 15 minutes at room temperature. The cells were rinsed twice with an ice-cold PBS and then incubated with 0.25% Triton X-100 in PBS for ten minutes. The cells then were rinsed with ice-cold PBS three times and incubated with fluorescein-isothiocyanate (FITC) for 15 minutes and then were rinsed twice with an ice-cold PBS and subsequently incubated overnight with 5µg/ml *rabbit polyclonal antibody anti- cfos* (Abcam, # ab83816) at 4_oC. The following day the cells were rinsed three times with PBS and then incubated with *goat polyclonal antibody to rabbit IgG* 1:1500 (ab 6717) for one hour at room temperature. The coverslips were mounted with fluoroshield mounting medium containing 4',6-diamidino-2-phenylindole (DAPI). The slides were inspected under a immunofluorescence microscope (Olympus BX51) with 200x magnification. Cells which were incubated with a secondary antibody only were used as a negative control. To assess the intensity of staining, IHC staining results were graded on a 0 to 3 scale: 0 being no staining; 1 weak nuclear staining faintly perceptible at high power; 2 is moderate nuclei staining; and 3 is the nuclei displaying strong staining [19].

Clonogenic assay

A clonogenic assay was used to assess the differences in reproductive ability (capacity of cells to split and produce progeny) between cells that have undergone exposure to radioiodine radiation and control/untreated cells. The treated and untreated cells were grown in twelve-well culture plates ($2.5x10_5$ cell/well) for 24 hours. The cells then were incubated for seven hours in 5% CO_2 at 37°C with HBSS containing 7.4 x 10_5 Becquerel/well of NaI-131 (BATAN, Indonesia) and 10mM HEPES (pH 7.3). The reaction was terminated by removing the NaI-131 containing medium and the cells were washed twice with cold HBSS. The cells were trypsinized, counted, and plated at densities of 500 and 1000 cells/well with growth medium in six-well culture plates. After that, the cells were grown for ten days. Macroscopic colonies formed were counted on the tenth day. Reproductive ability was calculated as the percentage of colonies in plates treated with NaI-131 compared to untreated ones.

Statistical analysis

Collected results are expressed as mean ± SD. Quantitative data were analyzed by ANOVA to compare means between groups followed by a Turkey test. Qualitative data were analyzed using Mann-Whitney, and $p<0.05$ was considered statistically significant. Correlation between c-Fos expression with effect of radioiodine was tested with Spearman's rho, and $p<0.05$ was considered significant.

III. RESULTS AND DISCUSSION

mRNA C-Fos expression by qRT-PCR

The expression of mRNA c-Fos was demonstrated in MCF7 cell. However, the expression was not detected in treated and untreated SKBR3 and HaCaT cells. The treatment with ATP, EGF or a combination of both for 30, 45 induced *m*RNA c-Fos in MCF-7 cell (Figure 1). ATP and EGF combined treatment for 30 minutes induced a c-Fos *m*RNA level of 23 ± 4 ($p<0.05$), and the values for ATP and EGF treatments were 1.17 ± 0.02 and 5 ± 2 respectively. The level of *m*RNA c-Fos started to reduce on 60 minutes of treatment. (Figure 2).

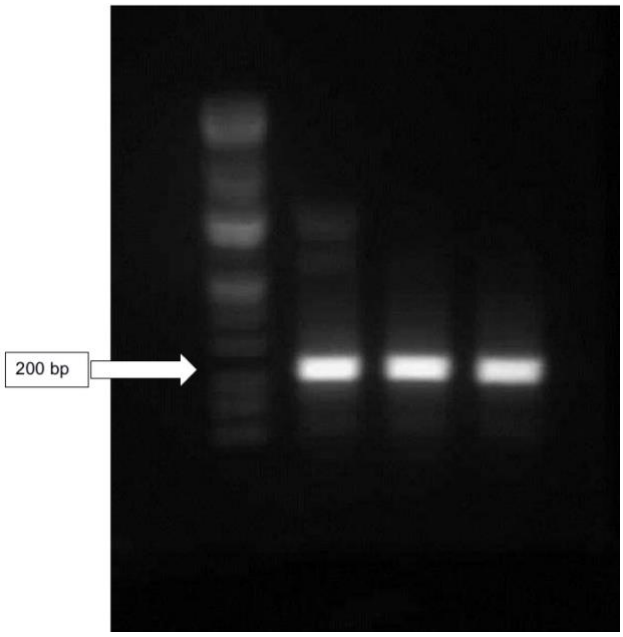

Figure 1: C-Fos gene is 231 base-pair in MCF-7 cells treated with ATP, EGF, and a combination of both for 30 minutes duration (right to left)

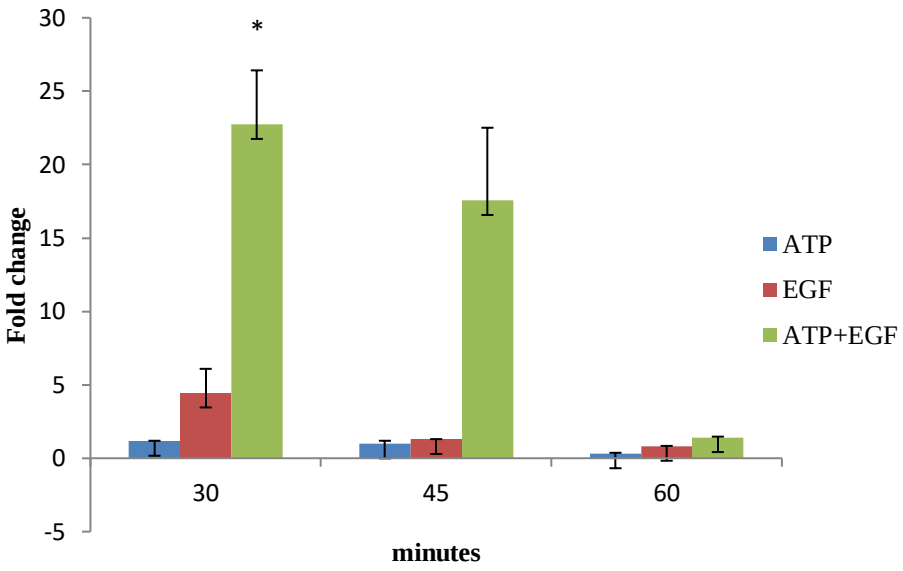

Figure 2: mRNA c-Fos expression in the MCF-7 cell line. The level of expression increases on 30 and 45 minutes of induction with ATP, EFG or a combination of the two. A combination of ATP and EGF increases c-Fos expression at a significantly higher level ($p<0.05$) compared to that of ATP or EGF alone. Duration of treatment longer than 45 minutes did not increase expression.

Immunocytofluoresence analysis of c-Fos protein expression

c-Fos protein was detected in the nuclei of MCF7 cells (Figure 3). Consistent with PCR results, SKBR3 and HaCaT cells did not express c-Fos protein. The c-Fos expression level increased 30 to 45 minutes after treatment and reduced after 45 minutes treatment. This same condition consistence with the c-Fos mRNA expression. Means of c-Fos increased after 30 minutes induction of ATP, EGF and combination of both are 3±0.5, 3±0, 3±0.5 respectively $p=0.04$. Means of c-Fos increased after 45 minutes induction of ATP, EGF and combination of both are 3±0.7, 2±0.5, 3±0 respectively $p=0.02$. Means of c-Fos after 60 minutes of induction were not different compare to control are 1±0.7, 1±0.5, 1±0.7 for ATP, EGF and combination of both respectively (figure 4).

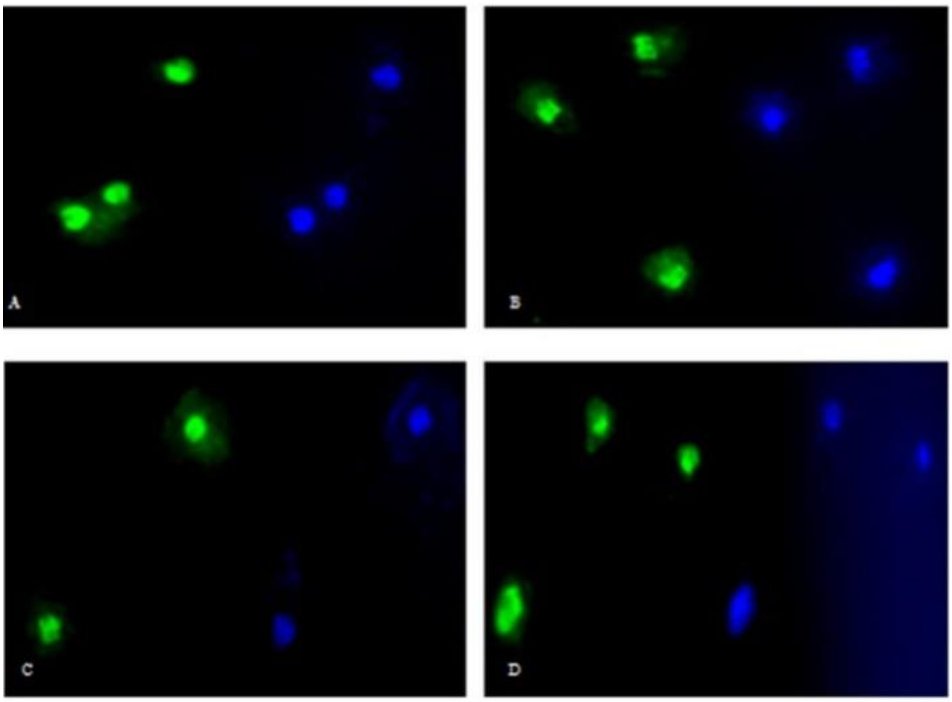

Figure 3 C-Fos protein expression shown at nuclei as green staining by FITC and a blue staining by DAPI at 200x magnification. The highest intensity of staining was shown on 30 minutes of treatment. C-Fos protein expression by ATP (A); EGF (B); and a combination of ATP+EGF (C); control (D). The expressions were persistent 30 to 45 minutes of treatment and started to reduce at 60 minutes.

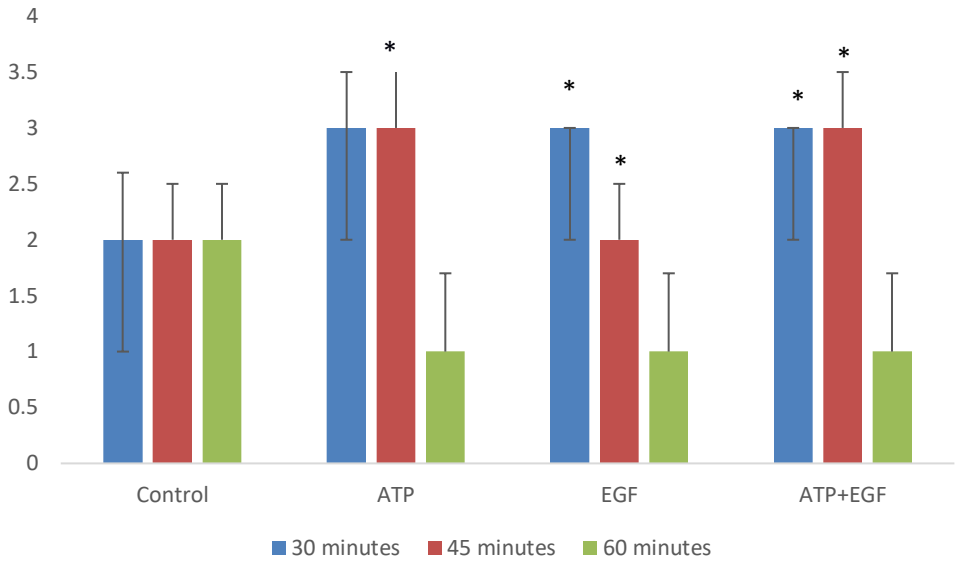

Figure 4: Analysis c-Fos protein expression in MCF7 cell line increased on 30 and 45 minutes all treatments significantly. The expression reduced on 60 minutes of all treatments.

Clonogenic assay after radioiodine radiation

Clonogenic assay was used to assessed cytotoxic effect of radioiodine on cell lines. The effect was calculated based on cell reproductive ability. Radioiodine exposure to the cells reduced the reproductive ability as 23 ± 5%, 53 ± 9% and 99 ± 4% for MCF7, SKBR3 and HaCaT respectively (Figure 5).

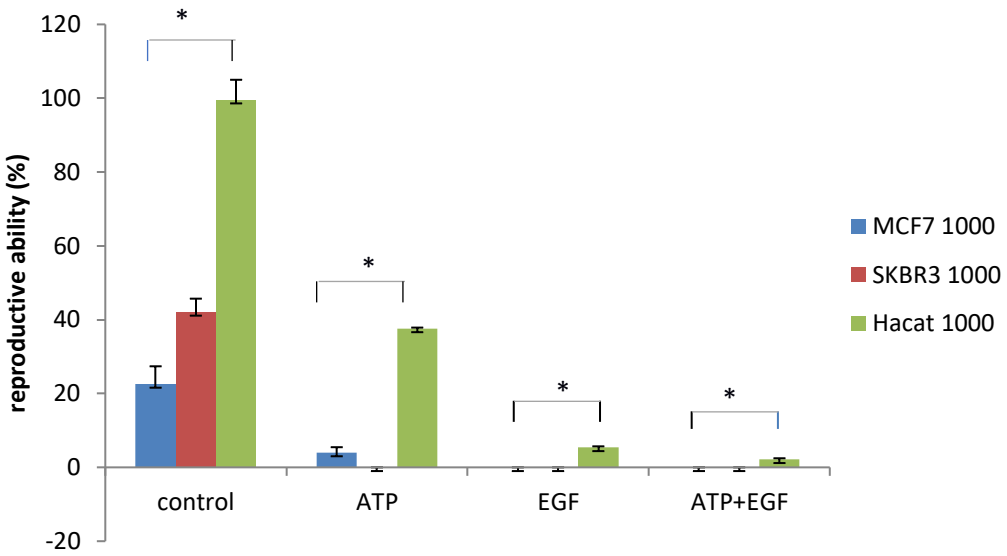

Figure 5: Reproductive ability after radioiodine exposure of three cell lines cultured in six well culture plates and treated with ATP, EGF and combination of ATP and EGF for 30 minutes prior to addition of $7.4x10^5$ Becquerel/well NaI-131. The control group received $7.4x10^5$ Becquerel/well NaI-131 alone. Radioiodine exposure reduced reproductive ability of MCF7 and SKBR3 cells significantly more than that of HaCaT cells ($p<0.05$). Treatment of MCF7 and SKBR3 with EGF or a combination ATP and EGF prior to radioiodine exposure resulted in a total discontinuation of reproductive ability $p<0.05$. Radioiodine exposure alone had almost no effect of on reproductive ability HaCaT cells, but ATP, EGF or a combination of both treatments prior radioiodine exposure resulted in a significant effect on the reproductive ability of the HaCaT cells ($p<0.05$).

Interestingly, radioiodine expose has very minimal effect on HaCaT cell reproductive ability. (Figure 5). Radioiodine exposure to MCF7 and SKBR3 cells reduced cell reproductive ability compared to HaCaT cells ($p<0.05$). ATP and/or EGF treatment along with radioiodine in SKBR3 cell line effectively eliminated the cell's reproductive ability. Furthermore, the reproductive ability of HaCaT cells after ATP, EGF or ATP/EGF combination treatment and radioiodine treatment were 38 ± 3%, 5 ± 0.3% and 2 ± 0.3% respectively. The treatments significant reduced of cell reproductive ability, $p<0.05$ (Figure 5).

We find an inverse correlation between the effect of radioiodine with mRNA and protein c-Fos levels in MCF-7 cells with $r = -0.906$ and $r = -0.973$ ($p < 0.05$) respectively. Treatment of ATP, EGF and combination of both increased the effect of radioiodine in suppressing the reproductive ability of the cells.

Estrogen receptors (ERs) play a pivotal role in the growth and maintenance of both normal breast cells and breast cancer cells. Estrogen receptors control transcription via direct and indirect DNA interactions. The direct pathway involves estrogen-activated ER binding estrogen-responsive elements and the indirect pathway involves liganded ERs which are bonded to DNA by interacting with activator protein-1 (AP-1). Heterodimers of c-Jun and c-Fos form AP-1 and it plays an important role in regulating breast cancer cell proliferation differentiation and proliferation and apoptotic process [15,20]. In our study, c-Fos expression was only found in the MCF7 breast cancer cell line. The expression did not detect in SKBR3 and HaCaT cell line. SKBR3 is HER2+ and ER-/PR- receptors. It may also be undetectable of c-Fos related to the absence of estrogen receptor [16].

NIS plays an important role to taken up iodine from extracellular and accumulated it intracellular [21]. Furthermore, NIS expression has an inverse correlation with c-Fos expressions [22]. Furthermore, the reproductive ability of cells after radioiodine exposure reduced more markedly in MCF7 cell compare to SKBR3 and HaCaT cells even though MCF7 does not express Natrium Iodide Symporter (NIS) as radioiodine transporter [23]. In this study, we found strong inverse correlation between c-Fos expression and reproductive ability of the cell after radioiodine exposure. We assumed, c-Fos increases cell sensitivity toward β radiation of radioiodine thru bystander effect that lead indirect cell death by produce the hydroxyl free radicals [8]. Besides of that molecule of iodine has effect as antiproliferative agent [24]. A report that the c-Fos/miR-22/MDC1 pathway plays a major role as a sensitizer in cancer therapy. C-Fos and miR-22 up-regulation causes a down-regulation of mediator DNA damage checkpoint protein 1 (MDC1). MDC1 plays a central role in the DNA damage response. It orchestrates double strand break (DBS) repairs and checkpoint activation that contribute to tumorigenesis. Thus, up-regulation of the c-Fos/miR-22/MDC1 pathway increases tumor sensitivity to anticancer drugs or radiation therapy

enhancing the therapeutic efficacy [25]. Further investigation is needed to uncover the role of c-Fos in apoptosis mechanism.

Furthermore, c-Fos expression is inducible. Fenretine (a synthetic retinoid) exhibits an ability to induce c-Fos, and the effect increases apoptosis rate in ovarian cancer cell lines [20]. Adenosine triphosphate (ATP) and epidermal growth factor (EGF) both moderately induce c-Fos expression, and the combination both ATP and EGF synergistically activates the extra-cellular signal-regulated kinase (ERK) pathway to increase c-Fos expression in MCF7 [26]. Our study shows that ATP and EGF alone or in combination significantly increase c-Fos protein, and this induction together with radioiodine treatments, synergistically increases cell death in MCF7 cell.

On the other hand, although SKBR3 cell did not express c-Fos but ATP and EGF treatments increased cell death after radioiodine exposure in the cell. We assumed the treatments stimulate the cells to activate the mitogen-activated protein kinase (MAPK) pathway. Increasing of the MAPK pathway inhibits DNA synthesis by expressing cyclin-dependent kinase inhibitor protein p21 [13]. We also find that treatment with ATP, EGF or combination of ATP and EGF increase the toxic effect of radioiodine in HaCaT cell line. Further studies are needed to elaborate the role of those treatments in cell death pathways. It seems different breast cancer subtypes have different pathways for cell death mechanisms.

Cell death mechanism in SKBR3 cells after radioiodine exposure is different from that in MCF7 cells. SKBR3 cell express NIS as transporter [22]. This transporter mediates radioiodine uptake [21]. A study reported that increasing NIS expression in ER-negative breast cancers boost the radioiodine uptake and susceptibility toward radioiodine radiation [3]. NIS enables radioiodine to be taken up and accumulated inside the cells. It emits β radiation which has a penetration of about 1 mm and hits DNA directly and water molecules to produce free radicals [8]. Depend on the dose, the radiation can produce DNA lesions such as double-strand breaks (DBSs) which appear to have a role cell cycle arrests and apoptosis [27,28]. Furthermore, besides damaging DNA directly, radioiodine exposure produces free radicals that caused indirect cell death. Interestingly, it has been found that HaCaT cells are

not affected by radioiodine, even at the levels of radioiodine uptake that are higher than in MCF7 cell [23]. The effect of radioiodine in that dose in HaCaT cell was un-significantly and this may be that normal cell more resistance toward radiation than the cancer ones.

IV. CONCLUSION

MCF7 cell line represented luminal A subtype expresses c-Fos and treatment of ATP and EGF increase the expression. C-Fos increases MCF7 cell sensitivity toward radioiodine exposure. A strong inverse correlation between the expressions of c-Fos and cell reproductive ability after radioiodine exposure. This result indicating a considerable potential of radioiodine for breast cancer treatment including luminal A subtype. Further studies are needed to elaborate the effect of radioiodine in different breast cancer subtypes.

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Subject: **Invitation to participate in WARMTH ISRT 2018**

Dear Dr. Aisyah Elliyanti,

On behalf of the World Association of Radiopharmaceutical and Molecular Therapy (WARMTH) it is my pleasure to invite you to the 2018 International Symposium on Radiopharmaceutical Therapy (ISRT) which will take place during November 17-20, 2018 in Helsinki, Finland

The organizing committee shall be more than pleased to extend all courtesies to you with regard to meals from 17-20th November, 2018. Delegates are responsible for their own accommodations and transportation. We look forward to your participation in person so that all other delegates have the opportunity to interact with you and enhance their knowledge in the field of Nuclear Medicine.

If you have any queries please feel free to get in touch with undersigned. Do visit our website https://warmth.org/icrt for more information including scientific agenda and visa information.

This letter is being issued to you to facilitate your getting a visa to participate in the above congress. It is our honor and privilege to welcome you to the above meeting and have your attendance.

Yours sincerely,

Dr. Irene Virgolini
President WARMTH
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